Medical University "Prof.Dr. Paraskev Stoyanov" – Varna
Faculty Medicine
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INFLUENCE OF SUBCHRONICALLY APPLIED LIGANDS OF CANNABINOID RECEPTORS ON LEARNING AND MEMORY PROCESSES IN OLFACTORY BULBECTOMIZED RATS

DISSERTATION
For the acquisition of the educational scientific degree
"DOCTOR (Ph.D.)"

Scientific supervisor: Assoc.Prof. Margarita Velikova, MD, PhD

Varna, 2022

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Varna, 2022

The dissertation has been discussed, approved, and proposed for official confirmation and validation at a conference meeting of the Department Council at the Department of Physiology and Pathophysiology, Faculty of Medicine, Medical University, Varna, Bulgaria

The dissertation consists of 146 standard pages, structured in 6 chapters, and includes a total of 26 figures, graphs, tables, and certificates. The literature includes a total of 418 sources in English.

The public seminar for the official defense of the dissertation will be held on April 8th, 2022.

Content:

Abbreviations used in the dissertation:

ECS - endocannabinoid system

EC - endocannabinoids

OB - bulbus olfactorius

OBX - olfactory bulbectomy

CB1, CB2 - cannabinoid receptors type 1 and type 2

Δ9-THC - Δ9-tetrahydrocannabinol

AEA - anandamide

2-AG - 2-arachidonoilglycerol

RIM - Rimonabant

SNK - test Student-Newman-Keuls

i.c.v. - intracerebroventricular

INTRODUCTION

The endocannabinoid system (ECS) is a lipid signal system that is functionally active from the earliest phases of brain development, active in the prenatal period, as well as after birth, and has an important role in the organization of the brain. ECS consists of endocannabinoids (EC), cannabinoid (CB) receptors, and enzymes, which regulate the production and decomposition of the EC.

By the late 80s and early 90s years of the 20th century the two cannabinoid receptors, CB1 and CB2 have been identified. Their pharmacologic effects are exerted mainly through the activation of Gi/o protein-associated membrane receptors. Even though both receptor types are members of the family G-protein- coupled receptors and are characterized with certain homology, they differ by their function and specificity of the cell expression (Koppel and Davies, 2008). Two endogenous cannabinoids (endocannabinoids) have been isolated in the 90-ies of the 20th century: N-arachydonoilethanolamin (AEA, anandamide) and 2-arachydonoilglycerol (2-AG), thus establishing their role as endogenous agonists of the cannabinoid receptors.

In recent years, special attention is paid to the use of cannabis extracts in medicine. Marijuana (*Cannabis sativa*) is a psychoactive plant with over 400 chemical compounds, 60 of which are phytocannabinoids, most of them with contradictory effects in the human organism. Most popular and well investigated amidst phytocannabinoids are Δ9-tetrahydrocannabinol (Δ9-THC) and cannabidiol. Δ9-THC is the major psychoactive cannabinoid, responsible for changes in mood, euphoretic feelings, and, cognitive disorders, which are the distinctive signs of cannabis effects on humans. The documented registry of marijuana usage includes anti-nociceptive, anti-inflammatory, antispasmodic, antiemetic effects, with predominantly application for recreational aims, thus, minimizing its medical application. There are

data for some phytocannabinoids, mainly cannabidiol, exerting favourable influence on various pathologic conditions. At the background of numerous publications for Δ9-THC and cannabidiol, most of the other plant cannabinoids are almost not investigated. The data of the action of synthetic substances, ligands of cannabinoid receptors, on behavioural reactions are also contradictory.

The compounds, modulating the ECS-activity, can damage and improve the various phases of memory formation by direct and indirect mechanisms. The altered ECS activity accompanies many psychiatric (depression, anxiety, schizophrenia) and neurodegenerative disorders (Alzheimer‘s disease, Parkinson‘s disease).

The main accent of the study, as an essential part of the present dissertation, is the evaluation of the influence of ligands of cannabinoid receptors on learning and memory disturbances, induced by olfactory bulbectomy. The experimental model olfactory bulbectomy (OBX) is based on surgical bilateral removal of bulbi olfactorii in rats. After performing OBX in rodents (rats or mice), different changes - behavioural, immune, endocrine, neurocellular, neurochemical, etc. are developed, similar to the ones established in patients with depression. The increased locomotor activity in an open space is one of the major-specific changes, observed after olfactory bulbectomy (van Riezen and Leonard, 1990). The exploratory activity of OBX-animals is also affected (Giardina and Radek, 1991; Vinkers, et al., 2009), the adaptation to the new environment is prolonged; memory deficits are also registered (Sieck, 1972; Primeaux and Holmes, 1999).

The OBX induced disturbances in exploratory and locomotor activity are specifically influenced by chronically administered antidepressants. In the last decade, the OBX is used not only as a model of depression but also as a model of Alzheimer’s disease, due

to the OBX-induced neurogenerative changes of the brain with consequent cognitive deficits.

The contradictory data on the role of cannabinoid receptors in the behavioural reactions of animals with models of depression (Segev et al., 2014; Kruk-Slomka et al., 2015; Haj-Mir- Zaian et al., 2017), as well as concerning cognitive functions, gave us the motivation to investigate the influence of subchronically applied ligands of cannabinoid receptors on some behavioural disturbances, developed in the OBX-animals 14 days after bulbectomy – exploratory behavior, learning, and memory.

Based on previous research with acute application of the ligands, and taking into consideration the fact that the antidepressive drugs exert their effect after long-term administration in the OBX-model, we decided to use in our experimental study different intervals of subchronic treatment (7 or 14 days) and different route of application: i.c.v. and intragastric. The changes of the tested parameters would give us a chance to evaluate how the ligands of the cannabinoid receptors would influence the depressive-like state in the OBX-model.

Our research aims to provide data about the involvement of cannabinoid receptors in the mechanisms of learning and memory disturbances and thus to contribute to the evaluation of CB receptors as a potential therapeutic approach in the therapy of depression and neurodegenerative diseases, accompanied by a cognitive deficiency.

AIM AND TASKS

Aim

To study the influence of subchronically applied CB-ligands (agonist and antagonist of cannabinoid receptors) on learning and memory processes in rats with experimental model olfactory bulbectomy (OBX).

Tasks

1. To study the influence of ligands of cannabinoid receptors on the exploratory behaviour and locomotor activity, learning, and memory, after a 7-day i.c.v. application in rats with experimental model olfactory bulbectomy (OBX).

2. To evaluate the effects of 14-day, intragastric application of CB1-receptor antagonist Rimonabant (SR-141716A) in OBX-rats (14 days before OBX, 14 days immediately after OBX, and 14 days after developing depressive-like state) on the exploratory behaviour and locomotor activity, learning, and memory.

3. To evaluate the importance of the time interval with Rimonabant application (SR-141716A) for the development of the exerted by the bulbectomy disorders of the exploratory behaviour and locomotor activity, learning, and memory in OBX-rats.

MATERIAL AND METHODS

Experimental animals

The experiments are performed on 294 male white rats, Wistar breed, weight 200-220 g. The rats are set in polypropylene cells with a free approach to food and water, permanent room temperature of 22±2ºC, regular light cycle 12-hour dark/12-hour daylight. The behavioural experiments are performed between 10.00 and 13.00 h.

Surgical procedures

Bilateral olfactory bulbectomy (OBX)

After anesthesia with Calypsol (50 mg/kg, i.p.), the experimental animals are fixed in stereotaxic apparatus (Stoelting, USA). The soft tissues on the head and periosteum are removed, and a puncture of the skull bones was drilled, left and right to the mean line. The coordinates of OB are determined by the Atlas of Pellegrino and Cushman (1967) for rats Wistar breed. The bulbectomy itself is performed by aspiration of bulbi olfactorii with a stainless steel needle, attached to a water pump. A 7-day recovery period for the animals is provided after the operation. The rats are adapted (handled) to the experiment in a daily regime. Sham-operated rats are subjected to the same surgical manipulations as the bulbectomized ones but without aspiration of the bulbs.

Implantation of leading cannulas in ventriculus ventrolateral dexter

The process of implanting the leading cannula is performed 3 days after olfactory bulbectomy. The animals are fixed after anesthesia in stereotaxic apparatus (Stoelting, USA). After removing the skull soft tissue, the skull bones are drilled, followed by implantation of a cannula in the right ventrolateral ventricle. The coordinates of ventriculus ventrolateralis dexter are determined according to the

Atlas of Pellegrino and Cushman (1967). The animals have a 7-day recovery period after the operation.

Drugs used

HU-210 (Tocris)

SR-141716A, Rimonabant (Sanofi)

Application of the drugs

HU-210 and SR-141716A are "ex tempore" dissolved in physiological solution and applied i.c.v. by using an injection cannula 1 mm longer than the leading one. The substances with ph=7.4 are introduced with a volume of 1 μl for 1 minute, whereas the injection cannula is left in its place for another 30 seconds.

HU-210, in a dose of 5 μg/1μl and SR-141716A (in a dose of 3 μg/1μl) are microinjected for 7 days (starting from the 15th day after OBX). The Sham-operated rats are injected with physiological solution, following the same procedure.

SR-141716A (Rimonabant, RIM)**,** dose 3mg/kg, is applied intragastrically for 14 days. The substance is dissolved „extempore" in physiological solution and introduced by the intragastric probe in a dose of 1 ml/100g. The used experimental animals are treated intragastrically with Rimonabant (RIM) for 14 days.

Verification

Anatomic verification is performed after the completion of the behavioural experiments. The verification is realized by microinjection of 1 μl 2 % methylene blue just before the procedure. The bulbectomy is verified macroscopically. The experimental data of animals to whom the drugs are improperly injected, or partial destruction of the bulbus is established (< от 80 %), are excluded from the final analysis of the results.

Behavioural methods

Method for examination of the exploratory behaviour and locomotor activity

The changes of the exploratory behaviour and locomotor activity are investigated according to the method of Kohler и Lorens (1978) with the apparatus Opto Varimex (Columbus Instruments, USA). The experimental chamber has dimensions 50x50x21 cm. The apparatus is constructed on a photocell principle - to register in arbitrary units the number of crossings of infrared light rays when the animal is moving, thus allowing a selective registration of the number of horizontal and vertical movements for a certain period.

The animal is located in the center of the experimental chamber and every crossing in the light ray, as a result of the movement of the animal, is automatically registered as an impulse. The movements of the animals are registered for 5 minutes (every minute and for a total of 5 minutes).

Methods to evaluate learning and memory

Two-way active avoidance test (apparatus shuttle box)

The test is performed in an apparatus shuttle box, after the method of Gozzani and Izquierdo (1976), modified by Petkov et al. (1993). Artificial light (electric lamp of 21 W, mounted on the top cover of each sector) is used for a conditioned stimulus. The light is switched on alternately in this sector in which the rat is not present at the end of the inter-session period. The conditioned stimulus (light) precedes the application of the unconditional (electric current) stimulus for 9 seconds and continues also in the time of action of the unconditional stimulus (21 seconds totally) for a single test. The electric current (0.5 mA; 50 Hz, 20-30V) is used as an unconditional stimulus. The electric current is activated on the metal lattice floor for 12 seconds in each session,

but only if no crossing of the rat from one to the other section of the apparatus is registered. The avoidance of the conditioned reflex is established only when the rat moves to the counter section of the apparatus in the time of action of the conditioned stimulus (light), i.e. during the 9 seconds before switching the electric current.

The test is performed following the scheme:
To achieve adaptation, a day before the experiment, each animal is located in the chamber of the apparatus and only light sessions are applied. The learning sessions are conducted in two consequent days. Each one of these two days includes 50 training sessions. The test for retention (memory) is performed on the 24th hour, after the second day of training. The light stimulus is applied for 9 seconds, followed by the electric current just for 2 seconds (something like "reminder"). The number of the avoidances for each training session (1-st, 2-nd day) and the memory test are registered as an index of learning and memory.

Passive avoidance test (step-through)
The passive avoidance test is conducted after the method of Buresova and Bures (1963). The apparatus has two chambers: one enlightened and a dark closed one. The learning includes a single training session. The animal is set on the platform in the enlighted chamber with an open door. After the animal enters the dark chamber, the door is closed and the electric current is switched on the floor net.
The memory test is performed on the 3rd and 24th hour after the learning session when each animal is again set in the enlighted chamber with an open door; the time of the rat, staying there is registered in seconds. As a criterion of achieved learning, the stay of the animal in the enlightened chamber should be not less than 180 seconds.

Statistic analysis of the results

The data are analyzed statistically by using a one-way and two-way ANOVA. The results from the step-through tests are analyzed using *the* chi-square test. All results are presented as mean values with their relative standard error ($X \pm S.E.M.$). To determine the considerable reliability of the differences between the corresponding groups we applied the post-hoc Student-Newman–Keuls (SNK), with minimum reliability $P \leq 0.05$.

RESULTS AND DISCUSSION

1. Influence of subchronically applied ligands of cannabinoid receptors on the exploratory behaviour and locomotor activity of OBX rats

1.1. Influence of HU-210 (CB-agonist) and SR-141617A (CB1-antagonist) after a 7-day i.c.v. application

The substances are applied independently i.c.v. in the right lateral ventricle of rats for 7 days, in doses 5 μg/1μl **(**HU-210) and 3 μg/1μl (SR-141716A). The tests in the Opto-Varimex apparatus are performed on day 21 after the operation (Sham or OBX), whereas the microinjection is performed 5 minutes before placing the animals in the chamber.
Two-way ANOVA (with repeating measures between both factors) is used to analyze the exploratory behaviour. These factors are "drug" with three levels (HU-210, SR-141716A, and saline), as well as "time" with five levels (1-st, 2-nd, 3-rd, 4th, and 5th minute). One-way ANOVA is used to analyze the total number of movements within the 5 minutes of investigation.

1.1.1. Influence of HU-210 and SR-141716A on Sham-operated rats

The post-hoc SNK test indicates that HU-210, applied i.c.v. in Sham-operated rats decreases the number of the horizontal movements at 1-st ($P \leq 0.001$), 2-nd ($P \leq 0.01$), 3-rd ($P \leq 0.0001$), 4-th ($P \leq 0.0001$), and 5-th ($P \leq 0.0001$) minute, compared to the Sham controls (Fig. 1).
SR-141716A increases the number of horizontal movements at 1-st ($P \leq 0.001$), 2-nd ($P \leq 0.01$), 3-rd ($P \leq 0.0001$), 4-th ($P \leq 0.0001$), and 5-th ($P \leq 0.0001$) minute, compared to the Sham controls (Fig. 1).

The post-hoc SNK-test indicates that HU-210 statistically decreases the total number of horizontal movements **(**$P \leq 0.0001$), compared to the controls. After the i.c.v application of SR-141716A, there is an increase in the total number of horizontal movements, compared to the controls ($P \leq 0.007$) (Fig. 2).

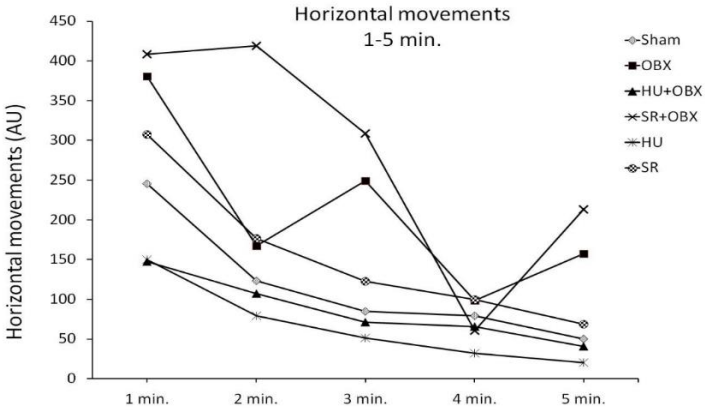

Fig. 1. *Influence of HU-210 and SR-141716A on the number of horizontal movements of Sham- and OBX-rats (7-day i.c.v. application) in 5 minutes of investigation (n = 7)*

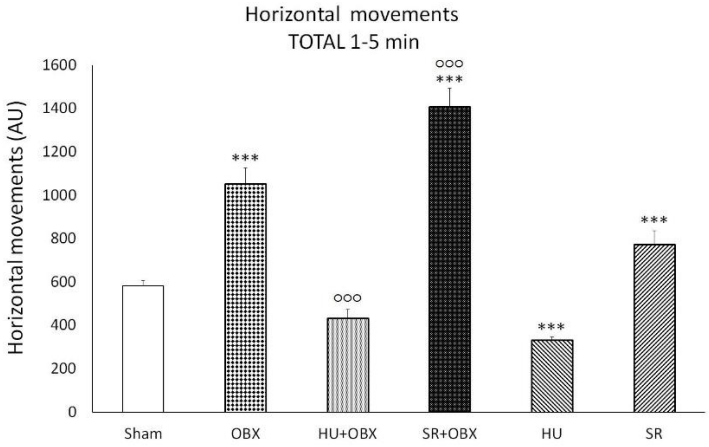

Fig. 2. *Influence of HU-210 and SR-141716A on the total number of horizontal movements of Sham- and OBX-rats (7-day i.c.v. application) in 5 minutes of investigation (n = 7); ***P ≤ 0.001 – compared to Sham-operated controls;* $^{000}P \leq 0.001$ *– compared to OBX-controls*

1.1.2. Influence of HU-210 and SR-141716A on OBX rats

An increase in the total number of horizontal movements in OBX-rats is registered during the 5 minutes of observation, compared to the Sham-operated controls ($P \leq 0.001$) (Fig. 2).

The analysis of the number of horizontal movements during every minute for the 5 minutes that HU-210 decreases the number of horizontal movements of OBX-rats at 1-st ($P \leq 0.0001$), 2-nd ($P \leq 0.01$), 3-rd ($P \leq 0.0001$), 4-th ($P \leq 0.001$) and 5-th minute ($P \leq 0.001$), compared to OBX-controls; the activity of the drug-treated did not differ considerably, compared to the Sham-controls.

HU-210 normalizes the habituation of OBX rats (demonstrated by a gradual decrease of the exploratory activity during the 5 min period).

SR-141716A, applied i.c.v. for 7 days, at the background of developed depressive-like state, increases the number of horizontal movements at 2-nd ($P \leq 0.0001$), 3-rd ($P \leq 0.04$), and 5-th min ($P \leq 0.05$), whereas decreases at the 4-th minute ($P \leq 0.001$). The analysis of the results shows that SR-141716A does not influence significantly the impaired habituation of OBX rats (Fig. 1).

HU-210 ($P \leq 0.0001$) significantly decreases the total number of horizontal movements, compared to the OBX-controls, but when compared to the Sham-controls, no considerable differences are established (P-NS). (Fig. 2). The normalization of the habituation and the locomotor activity of OBX rats under the influence of HU-210 could be considered a demonstration of an antidepressive effect.

SR-141716A increases the total number of horizontal movements, compared to the OBX-rats, $P \leq 0.004$ (Fig. 2).

1.2. Influence of Rimonabant (SR-141716A), applied intragastrically for 14 days before, immediately after, and 14 days after OBX), upon exploratory behaviour and locomotor activity of OBX rats

Number of horizontal movements for every minute and the total number of movements for5minutesd of observation

The number of horizontal movements upon 14 days RIM-application in Sham-operated rats is increased compared to Sham-controls: at 1-st ($P \leq 0.02$); 2-nd ($P \leq 0.003$); 3-rd ($P \leq 0.04$); 4-th ($P \leq 0.02$) and 5-th ($P \leq 0.05$) minute, and also the total number of movements for 5 minutes ($P \leq 0{,}001$) (Fig. 3, 4).

Horizontal activity in OBX-rats:

On the 14-th day after OBX, the number of horizontal movements is significantly increased at 1-st ($P \leq 0.001$); 3-rd ($P \leq 0.001$); 4-th ($P \leq 0.001$), and 5-th ($P \leq 0.001$); at the 2-nd minute no changes are

present (P-NS), the total number of horizontal movements is higher ($P \leq 0.001$), compared to the Sham- controls (Fig. 3,4).

Administration of Rimonabant 14 days before OBX (1-14d RIM, OBX)

RIM, applied 14 days before bulbectomy, increases the number of horizontal movements at 1-st $P \leq 0.001$); 2-nd ($P \leq 0.001$); 3-rd ($P \leq 0.001$); 4-th ($P \leq 0.001$); 5-th ($P \leq 0.001$) minute, compared to Sham-operated controls (Fig. 3). The total number of horizontal movements for the entire 5-minute period is increased as well ($P \leq 0.001$), compared to the Sham-controls (Fig. 3,4.)

We register an increase of the number of horizontal movements at 1-st ($P \leq 0.001$); 2-nd ($P \leq 0.001$) and 4-th ($P \leq 0.005$) in comparison with OBX_{14d}-controls; at 3-rd and 5-th minute no changes are registered (P-NS). The total number of horizontal movements for the 5 minutes dates increase as well ($P \leq 0.001$), compared to the OBX_{14d} -controls (Fig. 3,4.)

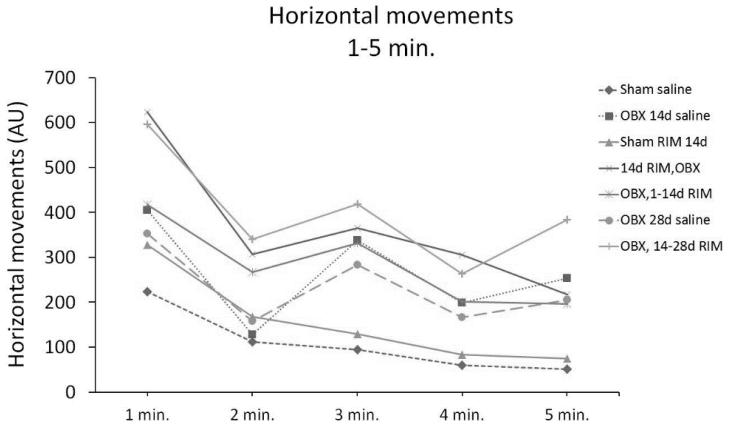

Fig. 3. *Influence of Rimonabant (SR-141716A; 3mg/kg) on the number of horizontal movements in OBX-rats during the 5 minutes of examination (14-day intragastric application, n=8)*

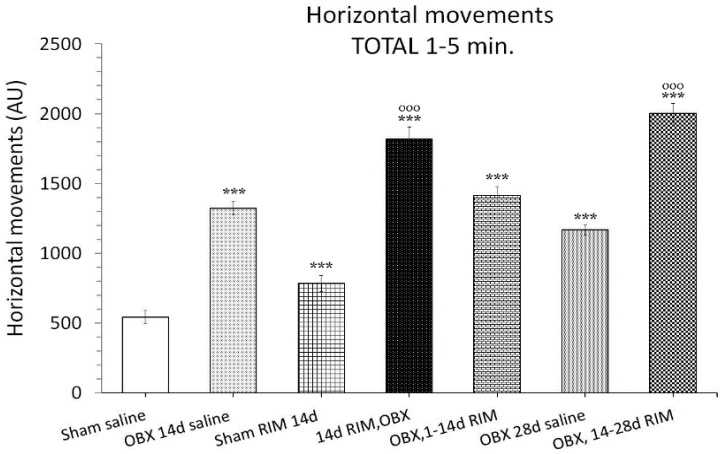

Fig. 4. *Influence of Rimonabant (SR-141716A; 3mg/kg) on the number of horizontal movements in OBX-rats during 5-minute period of examination (14-day intragastric application, n=8); ***P ≤ 0.001 – compared to Sham-operated controls;* $^{000}P \leq 0.001$ *– compared to OBX-controls*

Administration of Rimonabant immediately after OBX (OBX,1-14d RIM)

The post-hoc SNK test indicates that RIM, applied 14 days immediately after bulbectomy increases the number of horizontal movements at 2-nd ($P \leq 0.001$) and 5-th ($P \leq 0.04$) minute, compared to the control group (OBX_{14d}-saline), whereas at 1-st, 3-rd and 4-th minute no changes are observed (P-NS), (Fig. 3). Changes in the total number of horizontal movements compared to $OBX_{\mathbf{14d}}$ saline controls are not registered (P-NS) (Fig. 3, 4).

When compared to the Sham-operated controls, the OBX, 1-14d RIM-rats demonstrate a higher number of horizontal movements at 1-st ($P \leq$ 0.001); 2-nd ($P \leq 0.001$); 3-rd ($P \leq 0.001$); 4-th ($P \leq 0.001$); 5-th ($P \leq$

0.001) minueminutewell as higher total number of horizontal movements ($P \leq 0.008$), (Fig. 3, 4).
The results show that RIM applied immediately after OBX, does not influence statistically and reliably the changes of the horizontal activity, resulting after the extinction of bulbi olfactorii.

Administration of Rimonabant 14 days after OBX (at the background of developed depressive-like state (OBX, 14-28d RIM):

RIM applied from day 14-th until day 28-th after OBX, increased the number of horizontal movements on 1-st ($P \leq 0.002$); 2-nd ($P \leq 0.001$); 3-rd ($P \leq 0.03$); 4-th ($P \leq 0.02$); 5-th ($P \leq 0.01$), as well as the total number of horizontal movements for the 5 minutes ($P \leq 0.001$), compared to OBX-controls, tested 28 days after bulbectomy (OBX_{28d}-saline), (Fig. 3, 4).
Our study demonstrates that HU-210 applied i.c.v. for 7 days, normalizes the locomotor activity of OBX-rats during the 5-the 5 minutes ovation (decreases the number of horizontal and vertical movements, and the total number of movements does not differ significantly compared to the Sham-controls), whereas SR-141716A increases the locomotor activity when animals are placed in a new environment, compared to OBX and Sham-controls.
We were interested to evaluate the exploratory behaviour of the OBX rats, exposed to a new environment. The adaptation (habituation) to a new environment can be determined in a way of extinction of the orientation reflex with the time towards a repeating indifferent influence. Habituation is considered as one of the most elementary forms of learning, when the reduced investigation, as a function to multiple exposures to the environment, is accepted as a memory index (Thiel CM et al., 1998; Thiel CM et al., 1999). It is characteristic for the OBX-rats, that hyperlocomotion is accompanied by abnormal exploratory behaviour and impaired habituation to a new environment.

It is observed that the movements of the OBX-animals after they are placed in an open space, do not decrease gradually (which is seen in normal habitation), but the movements are activated after the 2-nd minute. It means that the animal can not orientate and can not adapt to the new environment. The administration of HU-210 in OBX-rats tends to a gradual decrease of the exploratory activity during the 5-minute 5 minutes application of SR-141716A at the background of an already developed depressive-like state does not show statistically reliable differences, i.e. the exploratory activity remains abnormal.

Based on these data we can conclude that HU-210 normalizes the exploratory behaviour of OBX-rats, whereas the CB1-antagonist SR-141716A further aggravated it by stimulating motor activity. The activation of CBRor blockade of CB1R in Sham-operated rats does not change considerably the exploratory activity (1- 5minute). After i.c.v.-application of CB-ligands, the activity gradually is reduced at a lower (with HU-210) or higher (with SR-141716A) level than those of the saline-treated controls. It is interesting to accent that the effects of HU-210 in the Sham-operated animals are limited, mainly on the locomotor activity, but do not influence considerably the habituation. The hyperactivity of OBX-rats in a new environment is a key symptom of a depressive-like state, which can be normalized by chronic use of antidepressant drugs. Due to this reason, we can interpret the results from the subchronic i.c.v.-treatment in OBX-rats with HU-210 (normalizing of the exploratory behaviour and locomotor activity, as well as the positive influence on memory processes, see below) in OBX-rats, like a tendency for the amelioration of the depressive-like symptoms. However, the antidepressive-like effect of HU-210 could be partially linked to the hypolocomotor effects, which are demonstrated in the Sham-operated animals.

The results of the positive influence of CBR-agonist on the exploratory and locomotor disorders, induced by OBX, and the

negative effect by the CB1 antagonist on the hyperlocomotion support the data that the dysregulation of ECS tends to a hyperlocomotor response after bulbectomy (Rodriguez-Gaztelumendi A. et al., 2009**;** et al., 2010).

The literature data about the effects of CB1-antagonists in animal models of depression are rather contradictory (Moreira et al., 2009).
Years ago, the successful use of CB1R-receptor antagonist Rimonabant to treat obesity, has been determined to provoke side effects, associated with an increased level of anxiety and manifestation of depressive symptoms, thus its usage has finally been stopped. The numerous studies on the CB receptor participation in a depressive-like state in experimental trials still cannot give confirmative results.
To add new data in this aspect, we expand our investigation of the effects of SR-141716A (Rimonabant, RIM) upon the exploratory activity of OBX-rats by using a different route of administration (intragastric), different duration (14 days), and time interval for the application.
Analyzing the results, we established that the time interval has a certain importance for the demonstration of the Rimonabant effects. RIM, applied 14 days before OBX (14d RIM, OBX), as well as 14 days after the manifestation of the depressive-like state (OBX, 14-28d RIM), increase the number of horizontal and vertical movements (1-5 minute), as well as the total number, compared to OBX-controls and Sham-operated controls, whereas the RIM-application immediately after bulbectomy (OBX, 1-14d RIM) does not show considerable changes in the number of movements compared to the OBX-controls. The data of exploratory behaviour indicate that as a result of a 14-day application of Rimonabant in all periods: before OBX (14d RIM, OBX), immediately after OBX (OBX, 1-14d RIM,) and 14 days after

revealing of a depressive-like state (OBX, 14-28d RIM), the habituation of OBX-rats remains impaired.

The incapability of the OBX-rats to adapt to the new environment is confirmed by the lack of moderate decrease of movements during the exploration of the small arena of the Opto-varimex apparatus in 5 minutes (lack of progressive reduction of the curve). The RIM application 14 days before the bulbectomy, as well as after the development of the depressive-like state (14-28d) results in stimulation of the locomotor activity, compared to the hyperlocomotor state, induced by OBX.

It is established, that the stable behavioural alterations, induced by OBX, among them the hyperlocomotion in an open environment, develops in the time interval 14 days after removal of bulbi olfactorii. Our study shows that exactly in these 14 days the application of RIM does not influence the locomotor activity. Quite interesting is the observation that upon various ways of subchronic SR-141716A-administration to OBX-rats (intracerebroventricular or intragastric), the stimulating effect on the locomotor activity is preserved. After a 14-day intragastric application of RIM to Sham-operated controls, we find an increase in the locomotor activity (total number of vertical and horizontal movements for 5 minutes). The habituation of the OBX-rats in the new environment, on the background of higher locomotor activity, is not significantly altered, compared to the saline-treated OBX-controls; in addition, no effect on habituation of Sham-operated controls (in fact, preserved normal) is registered.

The analysis of our results leads to a conclusion that the CB-receptors are involved in the development of the hyper locomotor activity of the OBX-rats. RIM, applied in a specific period (14 d before OBX and 14-28 d after OBX), leads to an additional aggravation of the OBX-induced disorder, whereas applied in the interval of development of behavioural OBX-syndrome (1-14 d), the hyperlocolocomotion is not considerably altered.

Our results are following the established by Gorzalka et al. relation between the disorders/blockage of endocannabinoid signaling and the incapability for acquaintance with the stress and adaptation in a new environment (Gorzalka BB et al., 2008).

2. Influence of subchronically applied ligands of cannabinoid receptors on learning and memory processes in OBX rats

2.1. Influence of HU-210 and SR-141716A after a 7-day i.c.v.-application:

2.1.1. Two-way active avoidance test (shuttle box)

The effects of HU-210 (5 μg/1μl) and SR-141716A (3 μg/1μl), microinjected 7 days in the right lateral ventricle, on the background of a depressive-like state, are examined on learning and memory processes by using two tests of avoidance (active and passive).

The post-hoc SNK-test indicates that HU-210 deteriorates learning and memory in the Sham-controls, i.e. considerably decreases the number of avoidances on 1-st day ($P \leq 0.04$), 2-nd learning day ($P \leq 0.01$), and on the retention test ($P \leq 0.006$) when compared to the saline-treated controls (Fig. 5. A, B, C).

SR-141716A improves learning and memory, with a statistically reliable increase of the number of avoidances on the 1-st learning day ($P \leq 0.04$), 2-nd learning day ($P \leq 0.03$), and on the retention test ($P \leq 0.04$), compared to the saline-treated controls (Fig. 5. A, B, C).

Worsening of the learning and memory processes, tested by a two-way active avoidance test is registered in OBX-rats. The SNS-test indicates that the number of avoidances reliably decreases during the time for learning: 1-st day ($P \leq 0.001$) and 2-nd day ($P \leq 0.001$), and in the retention test ($P \leq 0.001$), compared to the Sham-operated controls (Fig. 5 A, B, C).

We establish also that the HU-210 decreases the memory deficit in OBX-rats, statistically increasing the number of avoidances in OBX-rats on 1-st day ($P \leq 0.02$), 2-nd day ($P \leq 0.005$), and on the retention test ($P \leq 0.003$), compared to the OBX-controls, injected with saline.
SR-141716A, i.c.v.-applied in 7 days, decreases further the number of avoidances on 1-st day ($P \leq 0.05$) and 2-nd day ($P \leq 0.05$), and the retention test ($P \leq 0.01$), compared to the OBX-controls, injected with saline.
The parameters are lower and when compared to the Sham-controls a 1-st day ($P \leq 0.0001$), 2-nd day ($P \leq 0.0001$), and retention test ($P \leq 0.0001$) (Fig. 5 A, B, C).

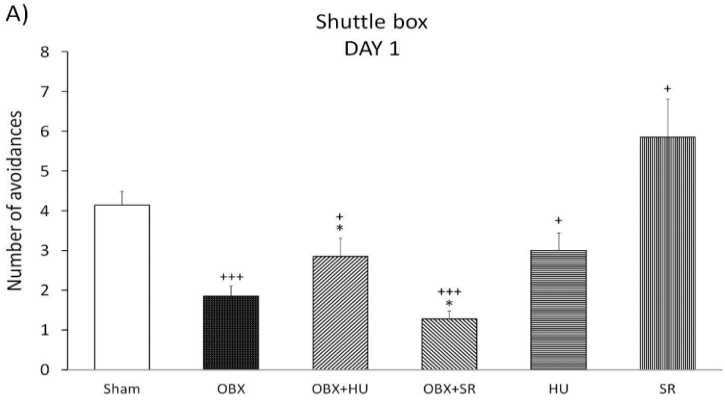

Fig. 5. (A, B, C). *Effect of HU-210 (5 μg/1μl) and SR-141716A (3 μg/1μl), i.c.v.-applied for a period of 7 days in OBX-rats, on the number of avoidances (shuttle box). A) Ist learning day; B) IInd learning day; C) retention test (n = 7).* $^{+}P \leq 0.05$; $P \leq 0.01$; $^{+++}P \leq 0.001$ *– compared to Sham-opetated controls;* $^{***}P \leq 0.001$ *– compared to OBX-controls*

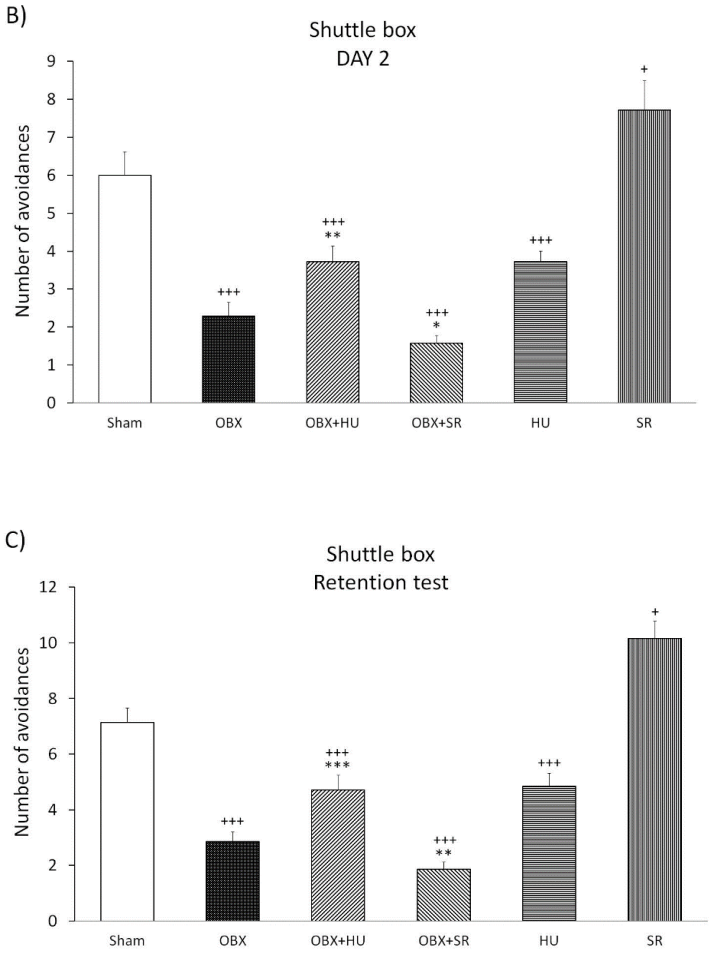

Fig. 5. (A, B, C). *Effect of HU-210 (5 μg/1μl) and SR-141716A (3 μg/1μl), i.c.v.-applied for a period of 7 days in OBX-rats, on the number of avoidances (shuttle box). A) I^st^ learning day; B) II^nd^ learning day; C) retention test (n = 7). $^{+}P \leq 0.05$; $P \leq 0.01$; $^{+++}P \leq 0.001$ – compared to Sham-opetated controls; $^{***}P \leq 0.001$ – compared to OBX-controls*

2.1.2. Passive avoidance test (step-through)

The method of passive escape with negative support (step-through) is used to determine the effects of HU-210 (5 μg/1μl) и SR-141716A (3 μg/1μl) on the memory, applied individually in the right lateral ventricle for 7 days and against the background of a depressive-like condition.

The i.c.v microinjection of HU-210 shortens the latent time during the retention tests on the 3-rd hour ($P \leq 0.01$) and on 24-th hour ($P \leq 0.006$), as well as decreases the percent of rats, reaching the criteria for learning on 3-rd (14%; $\chi^2 = 2.800$, $P \leq 0.05$) and 24-th hour (14%; $\chi 2 = 4.667$, $P \leq 0.02$), compared to the Sham- saline controls (57%, and 71% respectively) (Fig. 6,7).

The post-hoc SNK-test indicates that SR-141716A increases the latent time only for the retention test on the 3rd hour ($P \leq 0.05$), whereas for the test on the 24th hour no change is registered (P=NS). On the 3-rd hour the percent of rats, reaching the learning criteria, is increased to 71%, compared to 57% for the controls, whereas lack of change for the retention test (71%) is established compared to the Sham-controls, also 71% (Fig. 6, 7).

The bilateral removal of bulbi olfactorii worsens the parameters, measured by the test of passive avoidance: decreased latent time of the retention tests on 3rd hour ($P \leq 0.0001$) and 24-th hour ($P \leq 0.0001$), and also decreased to zero ($P < 0.001$) the percent of rats, reaching the learning criteria in comparison to the Sham-operated rats, on the 3-rd hour ($\chi^{2,} = 4.000$; $P \leq 0.05$) and on 24-th hour ($\chi^2 = 6.002$; $P \leq 0.02$) **(**Fig. 6, 7).

HU-210, applied 7 days, i.c.v. on the background of developed depressive-like state, prolongs significantly the latent time in the retention test on the 3-rd hour ($P \leq 0.03$) and 24-th hour ($P \leq 0.04$), and increases the percent of rats, reaching their learning criteria on the 24-th hour, 29 %, ($\chi 2 = 2.333$, $P \leq 0.05$), compared to OBX-rats (0%).

At the same time, the latent time shortened on the 3-rd hour ($\chi^2 = 2.800$, P = NS) and 24-th hour ($\chi^2 = 2.571$, $P \leq 0.05$), (Fig. 6, 7) compared to the Sham-saline controls.

After the SR-141716A application certain differences of the latent time for the OBX-animals are seen: reliable decrease on the 3-rd hour ($P \leq 0.05$); and increase on 24-th ($P \leq 0.01$), however, no change of the percent of rats, reaching the learning criteria on 3-rd and 24-th hour, compared to the OBX-controls, injected with saline (P-NS).

The comparison with the Sham-controls indicates that the OBX-animals, treated with SR-141716A, demonstrate shorter latent time on 3-rd hour ($P \leq 0.001$), and 24-th hour ($P \leq 0.001$); in addition, a decreased number of animals achieved learning criteria on the 3-rd hour ($\chi^2 = 5.600$, $P \leq 0.02$) and 24-th hour ($\chi^2 = 7.778$, $P \leq 0.01$), (Fig. 6, 7).

Our results show that the subchronically i.c.v-administered HU-210 improves learning and memory of OBX rats, whereas SR-141716A worsens the cognitive deficits, compared to the OBX-controls, treated with saline in both avoidance tests (shuttle box and step through). HU-210 shows a partial compensation of the memory deficits, resulting from the depressive state. The effects of HU-210 and SR-141716A on the OBX-rats are opposite and counter-related to those, established in the Sham-operated animals. HU-210, i.c.v.-applied to Sham-operated animals, worsens the learning and memory processes, whereas the SR-141716A improves them.

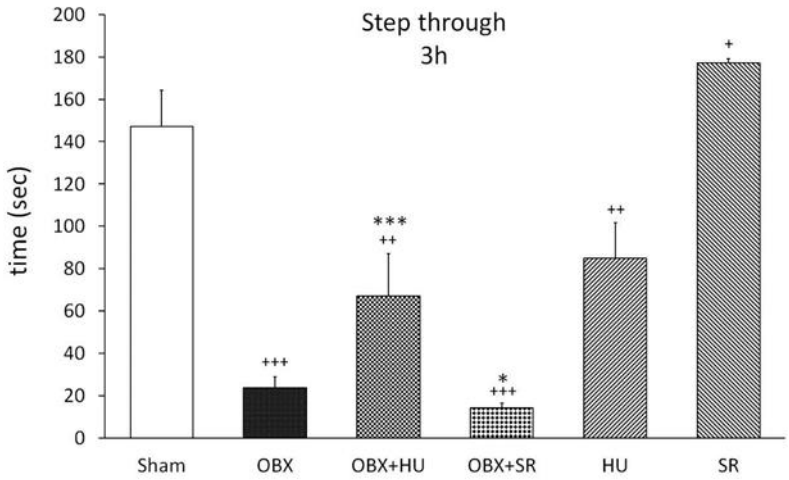

Fig. 6. *Effects of HU-210 (5 μg) and SR-141716A (3 μg), after a 7-day i.c.v.-application on OBX-rats, upon the latent time in a retention test on 3-rd hour (step through) (n = 7).* $^{+}P \leq 0.05$; $^{++}P \leq 0.01$; $^{+++}P \leq 0.01$; $^{+++}P \leq 0.001$ *– compared to Sham-operated controls;* $*P \leq 0.05$; $***P \leq 0.001$ *– compared to OBX-controls*

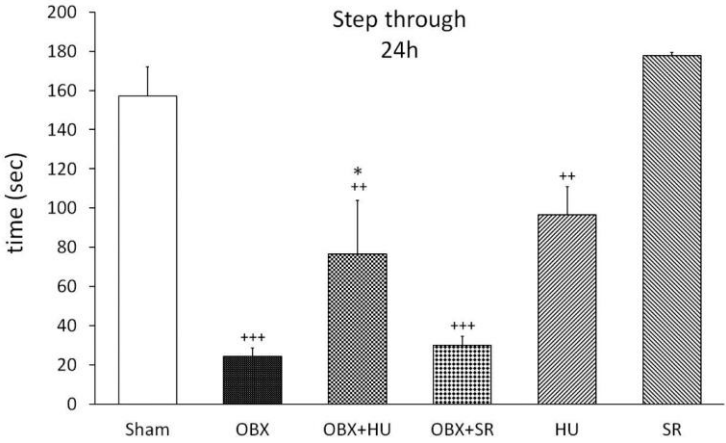

Fig. 7. *Effects of HU-210 (5 μg) and SR-141716A (3 μg), after a 7-day i.c.v.-application on OBX-rats, upon the latent time in a retention test on 24-th hour (step through) (n = 7).* $^{+}P \leq 0.05$; $^{++}P \leq 0.01$; $^{+++}P \leq 0.01$; $^{+++}P \leq 0.001$ *– compared to Sham-operated controls;* $*P \leq 0.05$ *– compared to OBX-controls*

2.2. Influence of Rimonabant (SR-141716A), applied intragastrically for 14 days before, immediately after,1-14d, and 14 days after, 14-28d OBX):

2.2.1. Two-way active avoidance test (shuttle box)

Rimonabant (Sham-RIM):

RIM increases the number of avoidances during the 1-st day ($P \leq 0.002$), 2-nd day ($P \leq 0.01$), and on the retention test ($P \leq 0.05$), compared to the Sham-controls (Fig. 8, 9, 10).

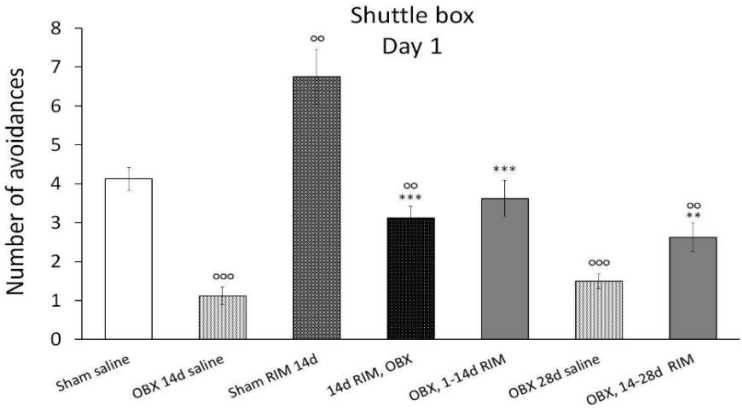

Fig. 8. *Effects of Rimonabant (3 mg / kg), intragastrically applied for a period of 14 days to OBX-rats on learning and memory (shuttle box, 1-st learning day) (n-8), ^{000}P <0,001, ^{00}P <0,01 – compared to Sham-controls, treated with physiological solution ∗∗∗ P < 0.001; ∗∗ P < 0.01 – compared to OBX-controls*

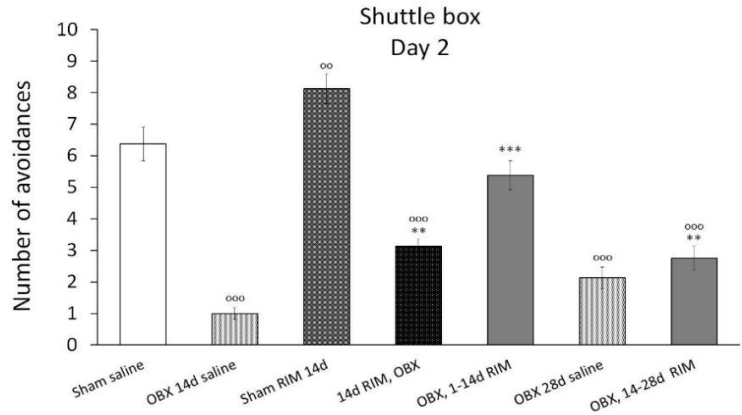

Fig. 9. *Effects of Rimonabant (3 mg / kg), intragastrically applied for a period of 14 days to OBX-rats on learning and memory (shuttle box, 2-nd learning day) (n-8), ^{000}P <0,001, ^{00}P <0,01 – compared to Sham-controls, treated with physiological solution ∗∗∗ P < 0.001; ∗∗ P < 0.01 – compared to OBX-controls*

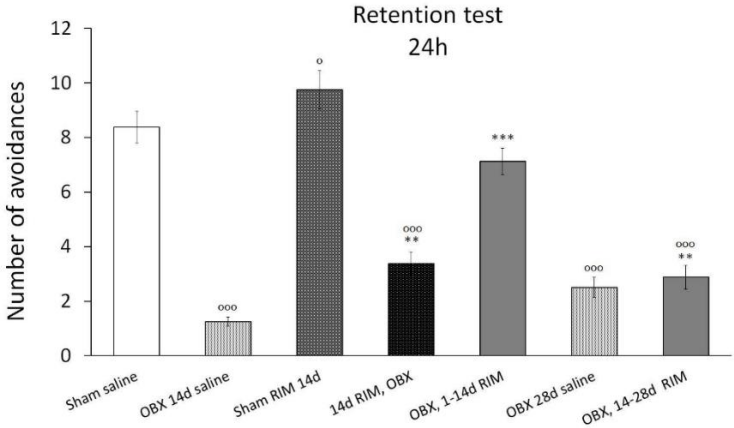

Fig. 10. *Effects of Rimonabant (3 mg / kg), intragastrically applied for a period of 14 days to OBX-rats on learning and memory (shuttle box, retention test) (n-8), $^{0}P < 0,05$, $^{000}P < 0,001$, – compared to Sham-controls, treated with physiological solution $***P < 0.001$; $**P < 0.01$ – compared to OBX-controls*

Changes in OBX rats

The learning and memory are seriously disturbed in bulbectomized animals (OBX_{14d} –saline), tested in the active avoidance test. The number of avoidances is decreased during the 1-st learning day ($P \leq 0.001$), 2-nd learning day ($P \leq 0.001$), and at the retention test ($P \leq 0.001$) compared to the Sham-operated controls (Fig. 8, 9, 10).

Rimonabant (1-14d RIM, OBX)

RIM, applied 14 days before OBX, tends to improve learning and memory, i.e. significantly increases the number of avoidances during the 1st and second training day, 1-st ($P \leq 0.001$) and 2-nd day ($P \leq 0.01$), as well as during the retention test ($P \leq 0.01$), compared to the OBX14d-saline controls. The comparison with Sham-controls, however, indicates lower results on the tests on the 1-st day ($P \leq 0.01$), 2-nd day ($P \leq 0.001$), and on the retention test ($P \leq 0.001$), (Fig. 8; 9; 10).

Rimonabant (OBX, 1-14d RIM):

In the group OBX, 1-14d RIM-rats treated immediately after bulbectomy, we RIM significantly improved learning and memory: the number of avoidances is increased during the 1-st ($P \leq 0.001$), and 2-nd days ($P \leq 0.001$), as well as at the retention test ($P \leq 0.001$), compared to OBX_{14d}-saline controls. There was no significant difference when comparing to the Sham-saline controls (during the 1-st and 2-nd day, and at the retention test) (Fig. 8, 9, 10), therefore we conclude that RIM prevents the development of bulbectomy-associated learning and memory disturbances.

Rimonabant (OBX, 14-28d RIM):

RIM, administered at the background of a depressive-like state (OBX, 14-28d RIM), shows statistically reliable improvement of the learning and memory processes, compared to the OBX_{28d}–saline controls, demonstrated by a higher number of avoidances for the 1-st ($P \leq 0.01$) and 2-nd day ($P \leq 0.01$), also for the retention test ($P \leq 0.01$), (Fig. 8, 9, 10).

Nevertheless, the comparison with Sham-operated controls shows that the parameters are worsening. The number of the avoidances decreases on 1-st day ($P \leq 0.004$), also on the 2-nd day ($P \leq 0.001$) and the retention test ($P \leq 0.001$), (Fig. 8, 9, 10).

When analyzing the parameters of the bulbectomized animals, tested 14 days after OBX ($OBX_{\mathbf{14d}}$) and 28 days after OBX ($OBX_{\mathbf{28d}}$), no significant differences in the tests on 1-st and 2-nd learning days, as well as the retention test, are founded (P-NS), (Fig. 8, 9, 10).

2.2.2. Passive avoidance test (step-through):

Rimonabant (Sham-RIM)

The RIM application prolongs the latent time at the retention tests on the 3-rd hour ($P \leq 0.05$) and 24-th hour ($P \leq 0.05$), thus tending to increase the percent of rats, reaching the criteria for learning on the 3-rd hour (75%; $\chi 2 = 1.657$; P=NS) and 24-th hour (75%; $\chi 2 = 1.657$; P=NS), compared to the Sham-controls (60%), treated by physiological solution. The latent time is prolonged, when compared to OBX_{14}-controls: at the retention tests on 3-rd hour ($\chi 2 = 4.000$; $P \leq 0.05$) and 24-th hour ($\chi 2 = 4.000$; $P \leq 0.05$), and the percent of rats, reaching the criteria for learning on 3-rd hour (14%; $\chi^2 = 2.800$, $P \leq 0.05$) and 24-th hour (14%; $\chi 2 = 4.667$, $P \leq 0.02$) is increased, (Fig. 11, 12).

Changes in OBX rats

The bilateral removal of bulbi olfactorii (OBX) induces a significant shortening of the latent time on the retention tests on 3-rd ($P \leq 0.001$) and 24-th hour ($P \leq 0.001$), as well as a decrease of the percent of rats (up to 0% on the 3-rd and 24-th hour), which reach criteria of learning, in comparison with Sham-operated rats - 3-rd hour ($\chi 2 = 9.643$; $P \leq 0.001$); 24-th hour ($\chi 2 = 9.643$; $P \leq 0.001$), (Fig. 11, 12).

Rimonabant (1-14d RIM, OBX)

Statistically, significant changes are not detected in the behaviour of OBX-rats treated with RIM before bulbectomy (1-14d RIM, OBX), compared to OBX_{14}-controls (on 3-rd and 24-th hour, P-NS)

Shorter latent time on 3-rd hour ($P \leq 0.05$) and 24-th hour ($P \leq 0.05$), and lower percent of rats, fulfilling criteria for learning on the 3-rd hour ($\chi 2 = 7.200$; $P \leq 0.01$) and 24-th hour ($\chi 2 = 4.866$; $P \leq 0.05$) are observed, in comparison to the Sham-controls, i.e. there is no influence on the memory deficits induced by the bulbectomy (Fig. 11, 12).

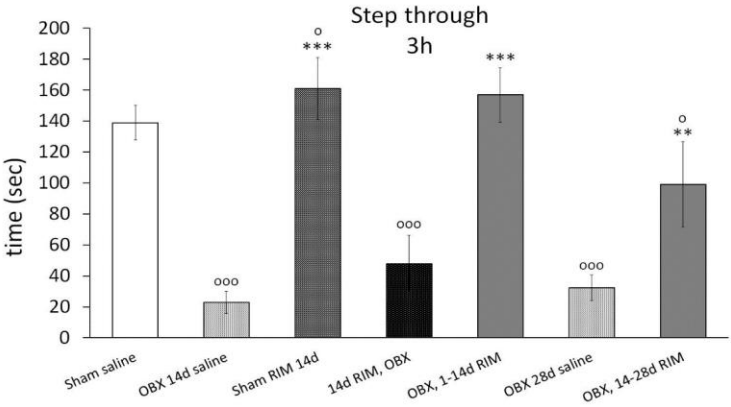

Fig. 11. *Effects of Ribanabant on memory (step through) after 14-day intragastral application of OBX-rats – test for memory on 3-rd hour (n =8)* ^{0}P *<0.05;* ^{000}P *<0.001 – comparison with Sham-controls, ** P <0.01, *** P<0.001 – comparison with OBX-controls*

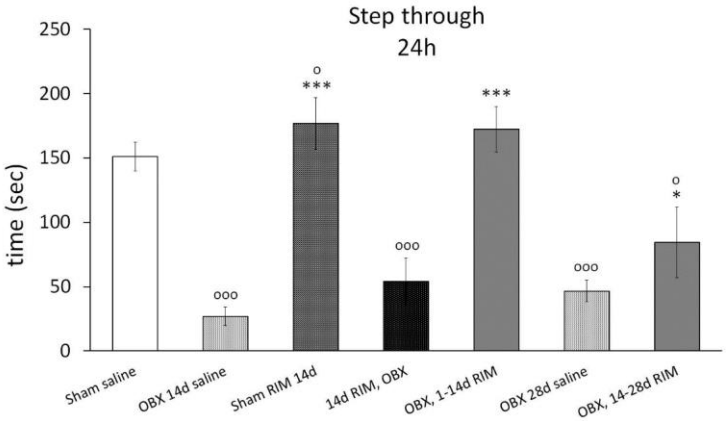

Fig. 12. *Effects of Ribanabant on memory (step through) after 14-day application of OBX-rats – test for memory on 24-th hour (n =8)* ^{0}P *<0.05;* ^{000}P *<0.001 – comparison with Sham-controls, ** P <0.05, *** P<0.001 – comparison with OBX-controls*

Rimonabant (OBX, 1-14d RIM)

RIM, administered immediately after OBX prolongs the latent time on 3-rd hour ($P \leq 0.001$) and 24-th hour ($P \leq 0.001$) and increases the percent of rats reaching the criteria for learning, as seen on the tests for retention on the 3-rd hour (62.5 %; $\chi 2 = 8.654$; $P \leq 0.01$) and 24-th hour (75%; $\chi 2 = 11.250$; $P \leq 0.001$) in comparison with OBX_{14}-controls (0%).

The parameters of the treated animals do not differ from those of the Sham-operated rats on 3-rd and 24-th hour (P-NS), which, indicates that the administration of RIM hinders the development of the OBX-induced memory deficit (Fig. 11, 12).

Rimonabant (OBX, 14-28d RIM)

RIM, administered after the development of a depressive-like state, 14-28 d after OBX, prolongs the latent time on 3-rd hour ($P \leq 0.01$) and 24-th hour ($P \leq 0.05$), and increases the percent of rats, reaching the criteria of learning, when tested on the 3-rd hour (37.5 %; ($\chi 2 = 3.692$; $P \leq 0.05$) and 24-th hour (37,5 %; $\chi 2 = 3.692$; $P \leq 0.05$), compared to OBX_{28}-controls (0%). The parameters of this group are worse, compared to the Sham-controls: shorter latent time on 3-rd hour ($P \leq 0.05$) and 24-th hour ($P \leq 0.05$), lower percent of rats, reaching the criteria of learning on the 3-rd hour ($\chi 2 = 7.200$; $P \leq 0.01$) and 24-th hour ($\chi 2 = 4.866$; $P \leq 0.05$), (Fig. 11; 12).

The main aim of the present study is, by using various models of application: i.c.v. and intragastric, in the conditions of the subchronic experiment (7 and 14 days), to evaluate the effects of ligands of cannabinoid receptors on learning and memory processes in rats with OBX model.

The CB-receptor agonist HU-210 improves the learning and memory deficits of OBX-rats with both tests performed, which is demonstrated

by an increased number of avoidances (active avoidance test, shuttle box), prolonged latent time, and an increased number of rats, reaching the learning criteria (passive avoidance test), however, the results of the Sham-operated controls are not achieved.

CB1-receptor antagonist SR-141716A has a negative influence on the OBX-rats in both tests (active and passive avoidance), demonstrated by a lower number of avoidances, shortened latent time, and lower number of rats, reaching the criteria of learning, i.e., the SR-141716A worsens learning, and memory of the OBX-rats, compared to the OBX-controls. However, in Sham-operated rats, HU-210 impairs learning and memory, whereas the application of SR-141716A improves the parameters in both tests.

The effects of HU-210 on the avoidance behaviour in rats are similar to those previously reported data of our research group, upon acute i.c.v.-injection of CB ligands (Marinov et al., 2013). Literature data show that the acute application of agonists of CB1-receptors impairs memory processes in various animal models (Abush H and Akirav I, 2013), while the treatment with SR-141716A prevents the manifestation of the memory disorders (Kruk-Slomka M et al., 2017). As for the influence of SR-141716A, we find that unlike the previous results from an acute treatment, where effects were present in the active avoidance test only, the subchronic 7-day i.c.v. injection to Sham-rats improved the rat's performance in both avoidance tests.

As far as the drug- or lesion-induced changes of the locomotor activity could influence mostly the active avoidance performance (Ogren и Stiedl, 2015), for evaluation of the effects of the CB-ligands, we used another test, for passive avoidance (step-through). During the performance of that test, longer stay in the light chamber of the apparatus depends on the suppression of the inherited habit to enter the dark section. The rats with a low level of locomotor activity would stay longer time within the lighted platform before moving to the dark section (longer latent time). As mentioned before, the locomotor

activity of OBX-rats is decreased under the influence of HU-210, whereas the SR-141716A demonstrates an increase.

An interesting fact of our study is that the CB-ligands exert opposite effects on the behaviour of Sham-operated rats in the avoidance tests, whereas the effects on the locomotor activity are similar in both groups. More details: HU-210 decreases the locomotor activity (Sham-operated and OBX-rats), worsens the memory (Sham-operated), and improves the memory deficit (OBX), whereas the SR-141716A increases the locomotor activity (Sham-operated and OBX-rats), improves the memory (Sham-operated), and worsens the performance in the active avoidance test (OBX).

The fact that each of the CB-ligands exerts the same locomotor effects in Sham-operated and OBX-rats, but act in an opposite way to influence the avoidance behaviour of the rats in both groups, allows us to presume that the changed locomotor activity does not considerably influence the drug effects on learning and memory.

Our results from the subchronic 7-day, i.c.v. microinjection of CB-ligands on OBX-rats, support the available literature data of anti-depressive and improving memory effect, associated with an increased ECS activity in different animal models with memory deficit (McLaughlin and Gobbi, 2012; Segev et al., 2014; Kruk-Slomka et al., 2015; Haj-Mirzaian et al., 2017; Kruk- Slomka and Biala, 2016).

Concerning the contradictory literature data about the effects of ligands of the CB receptors on learning and memory processes, our results support the negative effects of cannabinoid receptor activation on the memory of the experimental animals. The original data of our study indicate that the centrally applied CB ligands exert a modulating effect on learning and memory processes in olfactory bulbectomized rats. The increased activity of ECS with i.c.v.-injection of a CB-receptor agonist exerts an anti-depressive effect and improves cognitive processes evaluated in two avoidance tests.

To examine the influence of the endocannabinoid signalization in separate time intervals of OBX-syndrome development, we studied the effect of the CB1-receptor antagonist SR-141716A (Rimonabant), applied intragastrically for 14 days, before bulbectomy, immediately after bulbectomy (1-14 day), and 14 days after the operation (14-28 day) in OBX-rats, subjected to tests with active (shuttle box) or passive avoidance.

We establish that Rimonabant shows a tendency toward the improvement of the scores of Sham-operated rats in both tests for evaluation of learning and memory (passive and active avoidance). Our results confirm the data, that the application of SR-141716A, before the training sessions, improves the memory processes in rodents (Deadwyler SA et al., 2007; Lichtman AH, 2000; Terranova JP et al., 1996; Wolff MC, Leander JD, 2003).

We find out that Rimonabant applied 14 days before OBX, can not prevent the OBX-induced cognitive deficit in the passive avoidance test, but demonstrates a positive tendency in the active avoidance test. The CB1-receptor blockage after the development of a depressive-like state (14-28 days after OBX) ameliorated the deficits too. The results, revealing the ability of the CB1-antagonist to counteract the mechanisms, leading to the development of memory deficits in the avoidance tests need special attention. The behaviour of the animals treated immediately after bulbectomy (1-14 days after OBX), does not differ from those of Sham-operated controls.

The data of our investigation, regarding the influence of SR-141716A, applied by using various routes of administration in OBX-rats, as well as using different time intervals (before, during, and after the development of the OBX-syndrome) are again contradictory. The CB1-antagonist shed negative learning and memory effects in a 7-day i.c.v.-application, and in addition, a tendency to ameliorate the memory deficits during the 14-day intragastric application, whereas the most significant effect is established when the application is in the

interval immediately after the bulbectomy. These findings suggest that the disturbances of the ECS activity contribute to the memory deficits, accompanying the OBX-syndrome. In addition, the results of the subchronic, intragastric application of CB agonist, show that together with a stimulatory effect on the locomotor activity, SR-141716A e shows a tendency to counteract the development of the cognitive deficit, induced by the bulbectomy. Its application immediately after the bulbectomy normalizes the behaviour of the animals in both avoidance memory tests.

Conclusion

Based on the results of our experimental study we can conclude that the investigation of the influence of subchronically applied ligands of cannabinoid receptors in rats with a model of olfactory bulbectomy, contributes to establishing the role of CB-receptors in the development of memory deficits, accompanying the OBX model. The comparison of the effects between OBX- and Sham-operated rats allows a distinct evaluation of the physiologic importance of the CB-receptors for the cognitive processes. The ligands of the cannabinoid receptors in Sham-operated rats do not alter considerably the exploratory behaviour. After the i.c.v.-application the locomotor activity of sham-rats, placed in a new environment gradually decreases, although in a lower (HU-210) or higher (SR-141716A) level, than the one of the sham-controls. CB agonist HU-210 normalizes the exploratory behaviour (habituation) and the hyperlocomotion of OBX-rats. An increased locomotor activity after subchronic (i.c.v. or intragastric) application of SR-141716A (RIM) is registered in both Sham-operated and OBX-rats. The habituation of the OBX-rats in a new environment, with an accompanying higher locomotor activity, is not significantly influenced when compared to the OBX-controls.

The modulatory influence of ECS on the mechanisms of learning and memory is a complex one, and often demonstrated by contradictory results from the investigations. Our study presents additional data for the role of cannabinoid receptors in the brain structures, associated with the processes of learning and memory. Our results support the literature data for the participation of the endocannabinoid system in the development of memory deficits in olfactory bulbectomy.

There are reports that the activation of the CB1-receptors exerts anti-depressive, as well as pro-depressive effect, which explains the contradictory data in terms of the role of cannabis use for depression. We established that the CB-receptor agonist HU-210, applied

subchronically, i.c.v., shows an antidepressive-like effect, by normalization of the exploratory behaviour and locomotor activity, and by improving the learning and memory of rats with OBX-model of depression. In addition, the CB1-selective antagonist (SR-141716A), applied subchronically (i.c.v. or intragastrically), further aggravated the hyperlocomotion of the OBX-rats. An important finding of our study is that the effects of SR-141716A on learning and memory processes are opposite, depending on the route of administration. Whereas SR-141716A, applied subchronically i.c.v. in OBX-rats, worsens the memory deficits, upon intragastric administration it significantly improves the performance of OBX rats, investigated by the active and passive avoidance tests.

We provide also data that the blockage of the CB1-receptors in Sham-operated animals improves the learning and memory processes, tested by the avoidance tests, thus confirming the complex influence of the ECS on cognitive functions, which, in turn, rather often is altered by the moment condition of the organism.

We demonstrate the importance not only of the different routes of application but also of the time interval of administration, to manifest the effects of the subchronic, intragastric application of CB1-antagonist on the memory deficit of OBX-rats. It comes out that only upon administration in the time interval for development of a depressive-like state, the blockade of the CB1-receptors prevents the memory deficit, induced by the bulbectomy, whereas the application before and after this period tends to ameliorate the memory disturbances.

During the last decade, depressive conditions are one of the major socially important medical problems in the entire world. A big challenge for the healthcare system is to find medications, being able to treat both depressive and cognitive symptoms of the patients. The modulation of ECS activity seems to be a promising therapeutic aim in terms of depressive disorders and some neurodegenerative

disorders like Alzheimer's disease. Our results and conclusions underline the necessity of accumulating more data for the effects of the ECS-manipulation, thus widening the potential therapeutic approach to treat depression or Alzheimer's disease. The dissertation presents additional data to shed a light on the interrelation between cognitive and depressive symptoms, and also regarding the ECS influence on cognitive processes in disorders accompanied by a cognitive effect, (depression, Alzheimer's disease, etc). There is a necessity for new studies, new drugs, innovation strategies, thus, allowing us to determine which one of the specific treatments would be of value. Our modest expertise and an initial attempt to study the role of the cannabinoid receptors in learning and memory processes, specifically in memory deficits, is a step toward the knowledge of the cognitive functions of the brain.

CONCLUSIONS AND CONTRIBUTIONS

CONCLUSIONS

1. The modulation of the cannabinoid-receptor activity influences the learning and memory deficits, accompanying the model olfactory bulbectomy (OBX).

1.1. The subchronic (i.c.v.) application of CB-agonist HU-210 normalizes the disorders of the exploratory behaviour the locomotor activity in OBX-rats.

1.2. The CB1-selective antagonist (SR-141716A), subchronically applied (i.c.v. or intragastric) deteriorates the disorders of the exploratory behaviour and locomotor activity.

1.3. The subchronic i.c.v. microinjection of HU-210 shows a tendency to normalize learning and memory deficits of OBX-rats, investigated by tests for active and passive avoidance, whereas the SR-141716A worsens them.

1.4. The subchronic intragastric treatment with SR-141716A (Rimonabant) improves the parameters of learning and memory in OBX-rats.

2. The time interval is important for the manifestation of the Rimonabant effects on learning and memory processes in OBX-rats

2.1. Normalization of learning and memory deficits in OBX-rats, tested by avoidance methods, is registered only upon administration in the time interval immediately after OBX (1-14 days), whereas the administration before OBX and after the development of depressive-like state (14-28 day) shows a tendency to ameliorate the deficits.

3. The influence of the CB-receptor ligands on the behaviour of rats in avoidance tests depends on the experimental model.

3.1. In the OBX-model, with a different route of administration of the CB1-antagonist (SR-141716A), we find various effects on the

parameters of learning and memory: deterioration with i.c.v., and improvement with intragastric application

3.2. The SR-141716A influences positively the learning and memory processes of Sham-operated animals.

3.3. The activation of the CB-receptors (i.c.v.) shows opposite effects on the learning and memory in Sham-operated (impairs) and OBX-rats (improves).

CONTRIBUTIONS

Confirmatory contributions

1. Data are obtained, showing that the cannabinoid receptors participate in learning and memory processes, as well as in the development of the depressive-like state in OBX rats
2. It is established that the CB-1 antagonist (SR-141716A) shows a tendency for deepening of the depressive-like state, and its proper way for application is of certain importance for the manifestation of the effects on behavioural reactions of the animals

Original contributions

1. It is established that the ligands of the cannabinoid receptors exert various influences on the learning and memory in Sham- and OBX-rats
2. It is found that the activation of the cannabinoid receptors has a certain antidepressive effect in OBX-model
3. Data is obtained that the CB1-antagonist, with the intragastric application, improves the learning and memory processes in OBX-model
4. It is established that the time interval for application of the CB1-antagonist is important for influencing the behavioural deficits in OBX-model. The SR-141716A hinders the development of OBX-induced learning and memory disorders, with the intragastric application, immediately after OBX (1-14 days)
5. Our results contribute to the additional analysis of the role of the cannabinoid receptors in the learning and memory processes, as well as memory deficits, accompanying neurologic and psycho-mental diseases

Publications, associated with the dissertation

1. M.Velikova, **D. Doncheva,** R. Tashev. Effects of Rimonabant on active avoidance learning in bulbectomized rats, Journal of IMAB, Annual Proceeding (Scientific Papers), 2020 26(1), 2936-2941.

2. M.Velikova, **D. Doncheva,** R. Tashev. Subchronic effects of ligands of cannabinoid receptors on learning and memory processes of olfactory bulbectomized rats. Acta Neurobiologiae Experimentalis, 2020, 80(3) 286-296, **IF=1.529**

3. **D. Doncheva**, M. Velikova, R. Tashev. Effects of Rimonabant on learning and memory in olfactory bulbectomized rats. Notices of the Union of Scientists-Varna, 2'2018 vol. 13; 51-56.

Participation in scientific forums with presentations

Scientific forums in Bulgaria

1. **Doncheva D.,** Velikova M. Tashev R. Intracerebroventricular injection of cannabinoid CB1 receptor ligands modulates the exploratory activity of OBX rats. International scientific conference "Neuroscience, bioinformatics, microbiome and beyond", 17-19 September 2019, Bachinovo, Bulgaria, Abstract book. p.95.

2. Tashev R., **Doncheva D.,** Velikova M. Memory-modulatory effects of centrally administered cannabinoid receptor ligands in olfactory bulbectomized rats. Юбилейна научна конференция - 45-години МУ-Плевен 31.10 - 02.11.2019 г. *J Biomed Clin Res 12, 1- 2, 2019, p.110.*

3. **Doncheva D.**, Marinov M., Velikova M. Influence of the endocannabinoid system on memory processes. Closing Conference "Science in the Service of Society" - Varna, Oct.2020.

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1. D. Doncheva, M. Marinov, M. Velikova. Effects of subchronic ICV-treatment with cannabinoid CB1-receptor ligands on locomotor activity of rats with olfactory bulbectomy. 9th South-East European Conference of Chemotherapy for Infection and Cancer; 11-14 October 2018, Sarajevo, B&H

2. D. Doncheva, R.Tashev, M.Velikova. Effects of Rimonabant on locomotor and exploratory activities in olfactory bulbectomized rats. 11th South-East European Conference on Infection and Cancer and 31st Annual Assembly of International Medical Association Bulgaria; Plovdiv, 28-31 October 2021.

Annual Award "Young Scientist" for a presented poster in the Programme of 11-th South-East European Conference on Infections and Cancer, Joint Forum with 31-st Annual Assembly of IMAB, Plovdiv, Bulgaria

Young Scientists Annual Award: Dobrinka Doncheva
Supporting First Award

of 11-SEEC and 31-st IMAB Assembly, Пловдив, 28-31 окт. 2021

Poster: **"Effects of Ribonabant on locomotor and exploratory activities in olfactory bulbectomized rats"**: co-authors: Dobrinka Doncheva, Roman Tashev, Margarita Velikova (Medical University, Varna, Bulgaria)

Joint Forum SEEC-IMAB

11-th South-East European Conference
of chemotherapy, infections, and cancer
and
31-st Annual Assembly of International
Medical Association Bulgaria

28 - 31 October 2021,
Plovdiv, Bulgaria

Certificate: Young Scientists Annual Award
The Certificate is issued to confirm the **First Supporting Award** granted to:

Name: Dr. Dobrinka Doncheva

From: Dept. Physiology, Medical University, Varna, Bulgaria

Section: Medicine, Dental Medicine, Health Management, Varia (please, underline the corresponding section), for her attendance in the Scientific Programme of the Joint Forum: 11-th SEEC Infections and Cancer, and 31-st IMAB Annual Assembly, and she presented paper: **"Effects of Ribonabant on locomotor and exploratory activities in olfactory bulbectomized rats"** (co-authors: D. Doncheva, R. Tashev, M. Velikova

Rector of Medical University Plovdiv:
Prof.Dr. Marianna Murdjeva, MD, PhD, MHM
President of IMAB:
Prof.Dr. Krassimir Metodiev, MD, Ph.D., DScmed 30 October 2021